RICD 00-21

REMARKS

Claim 1 has been amended. Claims 1-7, 9-11, 13-15 and 18-20 are pending in the application. Applicant respectfully requests reconsideration of the claims in light of the amendments and the following remarks.

Claim 1 has been amended to more particularly point out a feature of the trimming operation in step c). The amendment to claim 1 is supported in the specification at, for example, page 37, last paragraph to page 38. No new matter has been added. The amendment to claim 1 presents claim 1 in better form for consideration on appeal and the entry of the amendment is respectfully requested.

Claims 1-7, 9-11, 13-15 and 18-20 stand rejected under 35 USC 103 (a) as being unpatentable over Burland (2000) in view of Smith et al. (1993). Insofar as these rejections may be applied to the amended claims, they are respectfully traversed.

An important feature of claim 1 is step c), the trimming step. According to the examiner, "Smith et al discloses the use of a best match algorithm in the trimming of known vector sequences from a polynucleotide sequence. ... Smith discloses that the user indicates the percentage of bases in the cloning vector sequence that must match the bases at the head or at the tail of a fragment sequence, and then the vector sequences are trimmed from that sequence. This appears to meet the limitations of the trimming step."

Applicant agrees with the examiner's characterization of Smith et al. However, applicant disagrees that Smith et al. appears to meet the limitations of the trimming step c) of claim 1.

One important feature of applicant's trimming step is the use of a best match type scoring algorithm which, during each scoring operation, **allows either a single nucleotide insertion or a single nucleotide deletion in the unknown raw nucleic acid sequence.** From the vague description given in Smith et al., it is unclear how "sequence regions" are matched against the

cloning vector fragment read from the disk. Smith et al. do indicate that a "percentage of bases" is specified by the user.

For discussion purposes, assume that the vector sequence read from the file is 100 bases long and that the user has specified that 75% (75 bases) must match. In the ideal case, the target fragment is perfectly sequenced by the laboratory instruments (i.e., the correct base-call is made for each nucleotide and there are no doubly-sequenced or dropped nucleotides). In this ideal case, when the vector is compared against the first 100 bases of the target sequence, then all 100 bases match. Assume next that the laboratory instrument called the 50th base in the target sequence incorrectly; then a match of 98% would result (well above the user specified criterion). If, however, the laboratory instrument either doubly-sequenced or dropped the 50th base in the target sequence, then all bases in positions 50 and above in the target sequence would match the vector sequence only by happenstance (a base match could still occur when there is a repeated base string in the vector). If the doubly-sequenced or dropped base occurred early in the target sequence, then most of the vector sequence would be compared against "erroneous" positions in the target sequence. Likely, this would result in a severely reduced matching percentage.

In the present invention, the ability to handle the double insertion (adding an extra base) or the drop of a base allows all bases in the target sequence downstream of the error to be compared against the correct positions in the vector sequence. Therefore, the claimed method of **allowing either a single nucleotide insertion or a single nucleotide deletion in the unknown raw nucleic acid sequence** is a significant improvement in the trimming operation. The single nucleotide insertion or deletion is not taught or suggested by Smith et al.

Smith et al. also do not consider the possibility for forward sense or reverse sense insertion of the nucleotide fragment into the cloning vector. In contrast, claim 1 recites that the scoring algorithm first compares a known positive 5' adapter sequence and a known negative 5' adapter sequence to the raw nucleic acid sequence and assigns a 5' trimming location to a position in the unknown raw nucleic acid sequence having the highest score and determines an **insertion orientation** according to the higher scoring of the known positive and negative 5' adapter sequences. By looking at both possible cases simultaneously, the claimed method is able

to determine the nucleotide fragment insertion direction. Smith et al. is silent on determining the insertion direction.

Furthermore, the method of claim 1 includes comparing a known positive confirmation sequence and a known negative confirmation sequence to the raw nucleic acid sequence and assigning a confirmation sequence location to a position in the unknown raw nucleic acid sequence having the highest score and determining a sequencing direction according to the higher scoring of the known positive and negative confirmation sequences. By comparing a portion of cloning vector known to be downstream of the insert against a portion of the target sequence after the second adapter is found, the claimed method can verify with some confidence that the entire nucleotide fragment was sequenced (along with some adapter sequence and cloning vector at the ends). Smith et al. do not teach or suggest any type of confirmation procedure or method for determining sequencing direction.

Still further, claim 1 recites a matching method not disclosed in Smith et al. The basic operation of "matching" two sequences must take into account the position of one (call it the "TOP" sequence) relative to the other (the BOTTOM sequence) and the congruency of the overlapping bases. For discussion purposes, assume that the bottom sequence is longer than the top sequence. As the top sequence is slid (to the right) past the bottom sequence, the first time overlap is encountered will be the rightmost base on the top overlapping with the leftmost base on the bottom. A score can be computed for that position depending on the congruency of the overlapping bases (just one overlapping base in this case).

As the top sequence slides further to the right, more and more bases will overlap at each slide position until all bases in the top sequence overlap bases in the bottom sequence. As the top sequence slides past the right end of the bottom sequence, fewer and fewer bases will overlap. The last overlapping base pair will be the leftmost top base and the rightmost bottom base. At each slide position, a score for that position will depend on the congruency of the overlapping bases. The method of claim 1 assigns a nucleotide paired with a **matching** nucleotide a highest value, assigns a nucleotide paired with an **unidentified** nucleotide an

intermediate value and assigns a nucleotide paired with a **mismatched** nucleotide a lowest value. Smith et al. simply do not address the type of scoring used.

As discussed above, the trimming operation recited in step c) of claim 1 includes at least four advantageous features that are not taught or suggested by Burland or Smith et al. Because of the many differences between claim 1 and the combination of Burland and Smith et al., claim 1 is allowable.

The remaining claims depend directly or indirectly from claim 1 and are allowable for that reason. In addition, the dependent claims recite further features not found in Burland or Smith et al. For example, claim 7 allows steps d)-f) to be performed at a preset later time. Claims 9 and 11 recite features of the first and second spreadsheets. It should be noted that Burland nowhere discloses the concept of the concise summary provided by the second spreadsheet.

In light of the above, claims 1-7, 9-11, 13-15 and 18-20 are in condition for allowance. Should there be any questions regarding this application, the examiner is invited to contact the undersigned attorney at the number shown below.

Respectfully submitted,

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